Clinical activity of FOLFIRI plus cetuximab according to extended gene mutation status by next-generation sequencing: findings from the CAPRI-GOIM trial

F. Ciardiello1,†*, N. Normanno2,†, E. Maiello3, E. Martinelli1, T. Troiani1, S. Pisconti4, F. Giuliani5, C. Barone6, G. Carteni7, A. M. Rachiglio2, V. Montesarchio8, G. Tonini9, D. Rizzi10, S. Cinieri10, R. Bordonaro11, A. Febbraro12, F. De Vita1, M. Orditura1, F. Fenizia2, M. Lambiase2, A. Rinaldi13, F. Tatangelo2, G. Botti14 & G. Colucci5

1Department of Clinical and Experimental Medicine ‘F. Magrassi’, Medical Oncology, Second University of Naples, Naples; 2Cell Biology and Biotherapy Unit, National Cancer Institute ‘Fondazione Giovanni Pascale’, Naples; 3Medical Oncology, Hospital Casa Sollievo Della Sofferenza–San Giovanni Rotondo (Foggia), San Giovanni Rotondo; 4Department of Medical Oncology, Hospital SS. Annunziata, Taranto; 5Department of Medical Oncology, National Cancer Institute Giovanni Paolo II, Bari; 6Department of Medical Oncology, University Hospital A. Gemelli, Rome; 7Department of Medical Oncology, Hospital ‘A. Cardarelli’, Naples; 8Department of Medical Oncology, Hospital Poli Occidentale, Castellaneta, Bari; 9Department of Pathology, National Cancer Institute ‘Fondazione Giovanni Pascale’, Naples, Italy

Background: Treatment with anti-epidermal growth factor receptor (anti-EGFR) monoclonal antibodies has been restricted to metastatic colorectal cancer (mCRC) patients with RAS wild-type tumors. Next-generation sequencing (NGS) allows the assessment in a single analysis of a large number of gene alterations and might provide important predictive and prognostic information.

Patients and methods: In the CAPRI-GOIM trial, 340 KRAS exon 2 wild-type mCRC patients received first-line FOLFIRI plus cetuximab. Tumor samples (182/340, 53.5%) were assessed by NGS to search for mutations in 22 genes involved in colon cancer.

Results: Objective responses in the NGS cohort were observed in 104/182 patients (overall response rate (ORR) 57.1%; 95% confidence interval (95% CI) 52% to 66.4%) with a median progression-free survival (mPFS) of 9.8 (95% CI 8.7–11.5) months. NGS analysis was successfully completed in all 182 samples. One or more gene mutations (up to five) were detected in 124/182 (68.1%) tumors within 14/22 genes for a total of 206 mutations. KRAS exon 2 mutations were identified in 29/182 (15.9%) samples, defined as wild type by local laboratory assessment. Frequently mutated genes were: TP53 (39.6%), KRAS exons 3/4 (8.8%), NRAS exons 2/3 (7.1%), PIK3CA exons 9/20 (13.2%), BRAF (8.2%). FOLFIRI plus cetuximab treatment determined ORR of 62.0% (95% CI 55.5% to 74.6%) with mPFS of 11.1 (95% CI 9.2–12.8) months in patients with KRAS and NRAS wild-type tumors. Conversely, ORR was 46.6% (95% CI 39.9–57.5%) with mPFS of 8.9 (95% CI 7.4–9.6) months in patients with KRAS or NRAS mutations. Similarly, the subgroup of patients carrying KRAS, NRAS, BRAF, or PIK3CA mutations showed a worse outcome, although this might be due to a prognostic effect.

Conclusions: This study demonstrates that NGS analysis in mCRC is feasible, reveals high level of intra and intertumor heterogeneity, and identifies patients that might benefit of FOLFIRI plus cetuximab treatment.

Key words: colorectal cancer, next-generation sequencing, cetuximab, KSA/NRAS, BRAF, PIK3CA

introduction

Molecular targeted agents with chemotherapy in treatment of metastatic colorectal cancer (mCRC) is an effective therapeutic option [1]. A subset of mCRC is dependent on epidermal growth factor receptor (EGFR) activation and treatment with anti-EGFR monoclonal antibodies (moAbs), such as cetuximab or panitumumab, in combination with standard chemotherapy (FOLFIRI or FOLFOX) is an effective therapeutic approach [2, 3]. Activating mutations in exon 2 of KRAS gene predict mCRC resistance to anti-EGFR therapies, bringing to the first molecular marker for clinical use in this disease [4]. Mutations in exon 2
(codon 12 or 13) of the KRAS gene account for 90% of KRAS mutations in mCRC [4]. Less-frequent KRAS mutations in exons 3 and 4, or NRAS mutations are markers of resistance to anti-EGFR moAbs in mCRC [5–8]. The European Medicine Agency restricted the use of anti-EGFR moAbs to mCRC patients whose tumors are wild type for both KRAS and NRAS genes.

In colorectal cancer, other genes encoding for key intracellular molecular transducers of EGFR activation, such as BRAF, PIK3CA, and PTEN, could be potentially associated with resistance to moAbs [8–10]. However, no firm conclusions can be drawn since studies of EGFR moAbs as first-line treatment of mCRC suggest prognostic rather than predictive effect of BRAF mutations, insufficient data are available for PIK3CA mutations [2, 3].

Since molecular alterations in different signal transduction genes play a role in resistance to anti-EGFR agents, optimization of these therapies in mCRC could require more extended molecular classification. Next-generation sequencing (NGS) techniques are powerful diagnostic tools that could allow the assessment in a single analysis of a large number of gene alterations for better patient’s selection treatment. NGS can provide information on inter- and intratumor heterogeneity that might allow a better stratification of patients eligible for personalized therapy. However, the feasibility of NGS in clinical diagnostics needs to be demonstrated.

The Cetuximab After Progression in KRAS wIld-type colorectal cancer patients (CAPRI)–Gruppo Oncologico dell’Italia Meridionale (GOIM) study explores the role of EGFR inhibition in second-line treatment of KRAS exon 2 wild-type mCRC patients after progression from first-line treatment with cetuximab; mCRC patients were treated with FOLFIRI plus cetuximab in first line and at progression were randomized to receive FOLFOX alone or FOLFOX plus cetuximab. Three hundred forty patients were enrolled in first line. The second-line part of the trial is currently ongoing.

Here, we report the results of NGS mutational analysis of a subgroup of patients enrolled in CAPRI trial. Correlation between mutational profile of these patients and efficacy of first-line FOLFIRI plus cetuximab therapy is also described.

patients and methods

study design and patient population

CAPRI is a nonprofit academic, open-label, multicenter study carried out by the GOIM cooperative group (Eudract number: 2009-014041-81; see supplementary Materials, available at Annals of Oncology online). Twenty-five centers participated to the trial (see supplementary Materials, available at Annals of Oncology online). Here, we present a retrospective, descriptive analysis of mutational profile carried out by NGS of a subset of patients enrolled in CAPRI trial. We also provide in a descriptive manner the correlation between mutational profile of these patients and efficacy of first-line FOLFIRI plus cetuximab therapy. The protocol was approved in each center by local independent Ethics Committee.

multiple gene mutation analysis by next-generation sequencing

Tumor samples were analyzed with the Ion AmpliSeq™ Colon and Lung Cancer Panel (Life Technologies) using Ion Torrent semiconductor sequencing. The panel contains primer pairs to analyze over 500 known mutations and eventually novel mutations in 87 hotspot regions of the following 22 genes: ALK, EGFR, ERBB2, ERBB4, FGFR1, FGFR2, FGFR3, MET, DDR2, KRAS, PIK3CA, BRAF, AKT1, PTEN, NRAS, MAP2K1, STK11, NOTCH1, CTNNB1, SMAD4, FBXW7, TP53 [11] (supplementary Figure S1, available at Annals of Oncology online). A detailed protocol is provided in supplementary Materials, available at Annals of Oncology online.

results

patients characteristics

From 15 July 2009 to 1 June 2013, 340 mCRC patients [intention-to-treat (ITT) population], with KRAS exon 2 wild-type tumors, determined by local laboratories, were enrolled in 25 Italian centers in CAPRI-GOIM trial (see supplementary Table S1, available at Annals of Oncology online). Patients received at least one infusion of FOLFIRI plus cetuximab with a median treatment duration of 12 cycles (6 months; range, 2–29 months) (see supplementary Table S2, available at Annals of Oncology online for toxicities).

multiple gene mutation analysis by next-generation sequencing

For 182 of 340 (53.5%) patients, FFPE tumor tissues, available in participating centers, were centrally collected and analyzed with Ion AmpliSeq™ Colon and Lung Cancer Panel. A 2% sensitivity threshold has been set for this panel following the results of a validation study [11]. High-quality DNA was extracted from all 182 samples thus multiple gene mutation assessment was possible in all these cases. As shown in Table 1A, no mutations in 22 tested genes were found in 58 of 182 (31.9%) cases; whereas one or more genes were mutated in 124 of 182 (68.1%) samples. Among the 22 genes, mutations were only found in 14 genes. The most frequently mutated gene was TP53 (72/182, 39.5%) (Table 1B). Two different co-existing mutations in TP53 gene were observed in seven cases. Forty-six KRAS gene mutations were detected, with one tumor sample having two different KRAS mutations. Therefore, KRAS gene was mutated in 45 of 182 (24.7%) cases. Surprisingly, KRAS exon 2 (i.e. codon 12 or 13) mutations were identified in 29 of 182 (15.9%) samples, although they were previously classified as having a KRAS exon 2 wild-type tumor by local laboratory measurement and, for this reason, enrolled in the current study. All KRAS exon 2 mutations were confirmed by using Sanger sequencing or the Therascreen KRAS RGQ kit. Mutations in exons 3 or 4 of KRAS gene were detected in 16 of 182 (8.8%) cases, while NRAS exons 2 or 3 mutations were found in 13 of 182 (7.1%) samples. All KRAS and NRAS mutations were point mutations in previously reported hot spot regions (data not shown). PIK3CA gene mutations occurred in 24 of 182 (13.2%) cases. Two co-existing additional PIK3CA mutations were detected in 2 of these 24 cases for a total of 26 mutations. Sixteen PIK3CA mutations were found in exon 9; whereas 10 mutations were located in exon 20. BRAF mutations were identified in 15 of 182 (8.2%) tumors, with 10 cases showing a V600E mutation, while other 5 samples were mutated in other regions of the BRAF gene. Less-frequent mutations were found in other nine genes, including MET (7/182,
Mutations in the EGFR and in the ERBB2 genes occurred very rarely (in two and in one cases, respectively). Multiple gene analysis by NGS identified several tumor samples with co-existing mutations in different genes (Table 2 and supplementary Table S3, available at Annals of Oncology online). In this respect, 206 mutations were detected in 124 samples. Thirty of 45 cases with mutated KRAS gene had concomitant mutations in other genes, including TP53, PIK3CA exons 9 and 20, BRAF, MET, PTEN, EGFR, or ERBB2. However, the presence of KRAS and NRAS mutations was mutually exclusive. The analysis by NGS revealed that, of 15 tumor samples with BRAF mutations, BRAF was the only mutated gene in three cases. In the other 12 cases, BRAF mutations co-existed with other mutations, including TP53, KRAS, and PIK3CA exon 9 and exon 20. A single PIK3CA mutation was found in only 5 of 24 samples with mutated PIK3CA gene. More frequently, PIK3CA mutations were detected together with other gene mutations, including TP53, KRAS, NRAS, and/or BRAF. In one case, a total of five different mutations were detected (PIK3CA exon 9 and exon 20, KRAS, ERBB2, TP53).

**clinical activity of FOLFIRI plus cetuximab according to multiple gene mutation analysis by next-generation sequencing**

We evaluated if NGS cohort of 182 patients was representative of entire ITT population of 340 patients. Clinical activity of FOLFIRI plus cetuximab was similar in this subgroup of patients when compared with ITT group (Table 3A). An overall response rate (ORR) of 57.1% and 56.4% were, respectively, reported in NGS and ITT populations. The median progression-free survival (mPFS) in NGS cohort of patients was of 9.8 months, whereas a median PFS of 9.9 months was observed in ITT population. We evaluated if the presence of gene mutations that could correlate with anti-EGFR moAbs efficacy in mCRC, such KRAS, NRAS, BRAF, and PIK3CA, affect the clinical activity of FOLFIRI plus cetuximab treatment. As illustrated in Table 3B, among the patients whose tumors were analyzed by NGS, 124 of 182 (68.1%) tumor specimen resulted wild type for both KRAS and NRAS genes. Treatment determined in the KRAS/NRAS wild-type cohort an ORR of 62.0%. Stable disease was reported in 35 of 124 (28.2%) patients, and 12 of 124 (9.7%) patients experienced progressive disease. The median PFS in the KRAS/NRAS wild-type group was of 11.1 months. On the contrary, mCRC patients with a tumor harboring KRAS or NRAS mutations achieved an ORR of 46.6% with a median PFS of 8.9 months. We evaluated the clinical activity of FOLFIRI plus cetuximab in patients whose tumors did not carry mutations in KRAS, NRAS, BRAF, and PIK3CA genes (Table 3C). FOLFIRI plus cetuximab treatment determined an ORR of 64.4% with mPFS of 11.3, whereas the ORR was 47.4% with mPFS of 7.7 months in patients with a mutation in any of these four genes. However, we must acknowledge that this difference could be due to a prognostic rather than predictive effect of BRAF and PI3CA mutations. No difference was found between the presence or the absence of TP53 gene mutations and clinical activity of FOLFIRI plus cetuximab (data not shown).

**Table 1.** (A) Twenty-two multiple gene mutation analysis in mCRC treated with FOLFIRI + cetuximab; (B) Number of cases (>2%) with most frequently gene mutations; (C) Number of cases (≤2%) with less-frequent gene mutations

<table>
<thead>
<tr>
<th>A</th>
<th>n/N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Analyzed for 22 gene mutations</td>
<td>182/340 (53.5%)</td>
</tr>
<tr>
<td>Wild type in all 22 gene analyzed</td>
<td>58/182 (31.9%)</td>
</tr>
<tr>
<td>Mutated at ≥1 of 22 genes analyzed</td>
<td>124/182 (68.1%)</td>
</tr>
<tr>
<td>Total mutations</td>
<td>206</td>
</tr>
<tr>
<td>Mutated genes</td>
<td>14/22</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>B</th>
<th>Gene</th>
<th>n (%) (N = 182 analyzed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TP53</td>
<td>72 (39.5%)</td>
<td></td>
</tr>
<tr>
<td>KRAS</td>
<td>45 (24.7%)</td>
<td>30 at codon 12 or 13 (16.5%); 16 at other (8.8%)</td>
</tr>
<tr>
<td>PIK3CA</td>
<td>24 (13.2%)</td>
<td>16 at exon 9 (8.8%); 10 at exon 20 (5.5%)</td>
</tr>
<tr>
<td>BRAF</td>
<td>15 (8.2%)</td>
<td>10 at codon 600 (5.5%); 5 at other (2.7%)</td>
</tr>
<tr>
<td>NRAS</td>
<td>13 (7.1%)</td>
<td></td>
</tr>
<tr>
<td>MET</td>
<td>7 (3.8%)</td>
<td></td>
</tr>
<tr>
<td>FBXW7</td>
<td>9 (4.9%)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>C</th>
<th>Gene</th>
<th>n (%) (N = 182 analyzed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EGFR</td>
<td>2 (1.1%)</td>
<td></td>
</tr>
<tr>
<td>CTNNB1</td>
<td>2 (1.1%)</td>
<td></td>
</tr>
<tr>
<td>FGFR3</td>
<td>2 (1.1%)</td>
<td></td>
</tr>
<tr>
<td>SMAD4</td>
<td>2 (1.1%)</td>
<td></td>
</tr>
<tr>
<td>ERBB2</td>
<td>1 (0.55%)</td>
<td></td>
</tr>
<tr>
<td>FGFR2</td>
<td>1 (0.55%)</td>
<td></td>
</tr>
<tr>
<td>PTEN</td>
<td>1 (0.55%)</td>
<td></td>
</tr>
</tbody>
</table>

3.8%) (Table 1C). Mutations in the EGFR and in the ERBB2 genes occurred very rarely (in two and in one cases, respectively).

Multiple gene analysis by NGS identified several tumor samples with co-existing mutations in different genes (Table 2 and supplementary Table S3, available at Annals of Oncology online). In this respect, 206 mutations were detected in 124 samples. Thirty of 45 cases with mutated KRAS gene had concomitant mutations in other genes, including TP53, PIK3CA exons 9 and 20, BRAF, MET, PTEN, EGFR, or ERBB2. However, the presence of KRAS and NRAS mutations was mutually exclusive. The analysis by NGS revealed that, of 15 tumor samples with BRAF mutations, BRAF was the only mutated gene in three cases. In the other 12 cases, BRAF mutations co-existed with other mutations, including TP53, KRAS, and PIK3CA exon 9 and exon 20. A single PIK3CA mutation was found in only 5 of 24 samples with mutated PIK3CA gene. More frequently, PIK3CA mutations were detected together with other gene mutations, including TP53, KRAS, NRAS, and/or BRAF. In one case, a total of five different mutations were detected (PIK3CA exon 9 and exon 20, KRAS, ERBB2, TP53).
NGS is a fascinating novel technique that allows obtaining information on mutational status of a large number of genes in a single analysis. In fact, this study is the first to demonstrate that mutational analysis of mCRC with NGS correlates with patients’ outcome.

Multiple gene mutation analysis of patients enrolled in the CAPRI trial was carried out with the Ion AmpliSeq™ Colon and Lung Cancer Panel [11]. Analysis with this platform was successfully conducted in all 182 samples retrospectively obtained from several different surgical pathologies (>20). Therefore, our findings suggest that NGS analysis might be feasible in a scenario resembling the clinical diagnostic setting. Nevertheless, NGS is a complex technique that should be carried out only in highly specialized centers with expert personnel. Dedicated external quality assessment (EQA) schemes for NGS should also be developed to ensure adequate quality of analysis.

The results of the CAPRI-GOIM trial confirmed the efficacy of the first-line combination of FOLFIRI plus cetuximab in KRAS exon 2 wild-type mCRC patients as previously reported [1, 12]. More recently, other less-frequent RAS gene mutations (KRAS exons 3 and 4 and NRAS exons 2, 3, and 4) are predictive markers of resistance to anti-EGFR therapies in mCRC patients [6–8, 13–15]. The analysis with the Ion AmpliSeq™ Colon and Lung Cancer Panel identifies the subgroup of mutant RAS patients that do not benefit of anti-EGFR moAbs treatment. Differential clinical activity of FOLFIRI plus cetuximab was observed in mCRC patients whose tumors were wild type for both KRAS and NRAS genes compared with patients whose tumors were carrying KRAS or NRAS mutations with ORR of 62.0% and mPFS of 11.1 months versus ORR of 46.6% and mPFS of 8.9 months, respectively. Similarly, patients carrying either KRAS, NRAS, BRAF, or PIK3CA mutations showed a worse outcome when compared with ‘quadruple wild-type’ patients. However, this could be due to prognostic effects of BRAF and PIK3CA mutations in mCRC rather than to the true predictive value of these mutations for FOLFIRI plus cetuximab treatment. The results of CRYSTAL, PRIME, and FIRE-3 trials do suggest that BRAF mutations are strong negative prognostic biomarkers for mCRC patients treated with chemotherapy alone (FOLFIRI or FOLFOX), chemotherapy plus anti-EGFR moAbs (cetuximab and panitumumab), and chemotherapy (FOLFIRI) plus bevacizumab [12–14]. Furthermore, PIK3CA mutation has no effect on the efficacy of FOLFIRI plus cetuximab in the FIRE-3 trial [14]. Therefore, no evidence sustains the use of BRAF and PIK3CA mutations for the selection of mCRC patients sensitive to anti-EGFR moAbs, although further studies should be carried out to explore this hypothesis.

A limit of the Ion AmpliSeq™ Colon and Lung Cancer Panel used in this study is that it does not cover exon 4 NRAS mutations. However, in this study, KRAS exon 3 or 4 and NRAS exon 2 or 3 mutations were detected in 15.9% of cases, a frequency similar to other studies, such as PRIME, PEAK, and FIRE-3 trials, in which no NRAS exon 4 mutations were as well identified [6, 13, 14].

Centralized, highly sensitive multiple gene analysis by NGS also enabled the identification of 29 additional patients with
KRAS exon 2-mutated tumors among the 182 cases that had been previously classified as KRAS wild-type exon 2 by local pathology laboratories. To our knowledge, this is the first report of a centralized and repeated analysis of KRAS exon 2 mutations within a clinical trial in mCRC patients treated with anti-EGFR drugs based on local pathology laboratory assessment. There are several potential explanations for the difference between original local laboratory assessment and centrally carried out NGS evaluation. The NGS technique that has been used in the present study has a sensitivity of 2%, which is much higher when compared with standard Sanger sequencing. In this regard, recent studies have suggested that low levels of KRAS mutations might lead to resistance to anti-EGFR mAbs [15]. NGS can also detect mutations that are not recognized by other methods used for KRAS testing in clinical practice. However, in the present study, only 6 cases had a percentage of KRAS exon 2 mutant alleles <10%, 8 samples had a percentage of mutant alleles between 10% and 20%, whereas 15 tumors carried >20% KRAS exon 2 mutant alleles (data not shown). Therefore, most of these mutations could be identified by Sanger sequencing. In addition, only one of the identified mutations is not included in the list of variants detected by commercially available kits (i.e. G13 V). Finally, we might have carried out NGS analysis on a different histology section from the one originally used by the local pathology laboratory. Nevertheless, a heterogeneous pattern of KRAS mutations is unlikely to occur in tumors with high frequency of mutant alleles, which were the majority of the cases. Taken together, these data suggest that false-negative results in KRAS exon 2 testing could occur in routine clinical practice, as also suggested by the results of European and Italian EQA schemes for KRAS mutation assessment [16, 17].

An important finding of the present study is that by using NGS it is possible to detect multiple gene mutations within the same tumor sample. Among the 22 genes that were studied, 14 were mutated with a total of 206 gene mutations being found in 124 of 182 samples, with an average of 1.67 mutations per sample. Genes whose mutations are considered mutually exclusive, such as BRAF and KRAS, were found mutated in the same tumor sample in some cases, as also recently reported [15]. In contrast, no sample was found to carry NRAS and BRAF mutations. Although the number of NRAS mutant cases in our study was low, this might suggest a different pattern of tumorigenesis for KRAS and NRAS. More importantly, identification of rare mutations as well as increased knowledge on the comprehensive mutational profile of each individual tumor might improve development of personalized medicine in mCRC. A number of novel agents targeting molecular alterations in genes involved in tumor growth and progression are in clinical development, NGS screening of a wide array of genes might facilitate the identification of mCRC patients that might be enrolled in trials with new drugs. Several initiatives are ongoing in including a European database collecting the results of the different NGS-based screening programs would help to accelerate the progress of personalized medicine in mCRC.

In most cases, we found that coexisting mutations occurred at different frequencies in the involved genes. Because the frequency of the mutation is directly related to the number of tumor cells that carry the mutant gene, these findings suggest that tumor clones with different mutational profile might be present in the same sample. These results suggest that, in agreement with recent findings in other tumors [18], CRC is characterized by intertumor heterogeneity, since we could recognize subgroups of tumors carrying specific molecular alterations, as well as by intratumor heterogeneity due to the presence of cancer cells with different molecular alterations in the same sample. Further studies will be needed to investigate these hypotheses and how tumor heterogeneity could affect response to therapy with anti-EGFR drugs.

In summary, the results of present study confirm the efficacy of cetuximab in combination with FOLFIRI in first-line treatment of mCRC patients with KRAS and NRAS wild type tumors. To our knowledge, this is the first trial in which...
molecular characterization carried out with multiple gene NGS-based technique has been correlated with clinical outcome in mCRC patients treated with an anti-EGFR moAbs.

acknowledgements

FC and NN thank the Associazione Italiana per la Ricerca sul Cancro (AIRC).

funding

This work has been supported by Associazione Italiana per la Ricerca sul Cancro (AIRC) (no grant number).

disclosure

The authors have declared no conflicts of interest.

references

7. Stintzing S, Jung A, Rossius L et al. Analysis of KRAS/NRAS and BRAF mutations in FRIE-3: a randomized phase III study of Folfiri plus cetuximab or bevacizumab as first-line treatment for wild-type (WT) KRAS (exon 2) metastatic colorectal cancer (mCRC) patients. Eur J Cancer 2013; 49(suppl 3); LBA17.